

Mitochondrial DNA Sequence Variations and Genetic Relationships among Korean *Thais* Species (Muricidae: Gastropoda)

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ABSTRACT

Thais Röding, 1798, commonly known as rock-shell, is among the most frequently found gastropod genera worldwide on intertidal rocky shores including those of Japan, China, Taiwan and Korea. This group contains important species in many marine environmental studies but species-level taxonomy of the group is quite complicated due to the morphological variations in shell characters. This study examined the genetic variations and relationships among three Korean *Thais* species based on the partial nucleotide sequences of mitochondrial *cox1* gene fragments. Phylogenetic trees from different analytic methods (maximum parsimony, neighbor-joining, and maximum likelihood) showed that *T. bronni* and *T. luteostoma* are closely related, indicating the most recent common ancestry. The low sequence divergence found between *T. luteostoma* and *T. bronni*, ranging from 1.53% to 3.19%, also corroborates this idea. Further molecular survey using different molecular marker is required to fully understand a detailed picture of the origin for their low level of interspecific sequence divergence. Sequence comparisons among conspecific individuals revealed extensive sequence variations within the three species with maximum values of 2.43% in *T. clavigera* and 1.37% in both *T. bronni* and *T. luteostoma*. In addition, there is an unexpectedly high level of mitochondrial genotypic diversity within each of the three Korean *Thais* species. The high genetic diversity revealed in Korean *Thais* species is likely to reflect genetic diversity introduced from potential source populations with diverse geographic origins, such as Taiwan, Hong Kong, and a variety of different coastal regions in South China and Japan. Additional sequence analysis with comprehensive taxon sampling from unstudied potential source populations will be also needed to address the origin and key factors for the high level of genetic diversity discovered within the three Korean *Thais* species studied.

Keywords: *Thais*, Korean rock-shell, mitochondrial *cox1* variation, Muricidae

INTRODUCTION

Thais Röding, 1798 (commonly known as rock-shell) is one of the most frequently found genera of gastropods worldwide on intertidal rocky shores including those of Japan, China, Taiwan, and Korea. The genus contains about a hundred species names (Houart and Gofas, 2010), some of which includes predatory species that cause serious damage to the oyster culture industry by drilling through the oyster shells (Clench, 1947; Brown and Richardson, 1988). The species of this genus also play an important role in the marine macrobenthic community. They have often been used as an effective indicator species in many marine environmental studies

related to human-induced pollution, such as that from organotin compounds (Shim et al., 2000; Hung et al., 2001; Tang and Wang, 2009) and long-term exposure to heavy metal contamination (Rubio et al., 1993; Han et al., 1997; Blackmore and Wang, 2004).

Despite their significance in aforementioned environmental studies, the species-level taxonomy of this group is quite complicated due to morphological variations in shell characteristics (Tan and Sigurdsson, 1996). Indeed, the morphological features used most frequently for species identification, including size, shape and the absence or presence of blotches on the nodules of shell surfaces and shell apertures, are highly variable depending on the local environment (Hayashi,

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1999; Tan and Liu, 2001). Owing to the substantial variability in shell characteristics, species identification relying on shell morphology alone often leads to erroneous results creating many synonyms. As a result, the associated nomenclature for *Thais* species is unusually complicated (Clench, 1947; Tan, 1995).

In Korea, *Thais* species have been included as part of a species checklist in the faunal report on mollusks. Earlier studies, including some encyclopedias of Korean shells, relied entirely on the shell characteristics without careful appraisal of species identity (Choe, 1992; Lee and Min, 2002; Min, 2004). A taxonomic investigation of Korean muricid species based on a morphological comparison of shell and radula characteristics showed that three *Thais* species are very abundant in Korean coastal areas (Choe and Park, 1997): *T. clavigera* (Küster, 1858), *T. bronni* (Dunker, 1860), and *T. luteostoma* (Holten, 1803). In contrast to taxonomic assessments by most earlier authorities, these species are not very morphologically discernible from each other because their shell morphology is greatly variable, creating a diverse array of local morphotypes according to their geographic origins. Therefore, there is considerable taxonomic confusion with regard to species-level taxonomy in this group.

The utility of molecular markers in modern taxonomy has been growing rapidly as a reliable tool for examining the phylogenetic relationships in different levels of taxonomic groups and species identification. As in many other animal groups, molecular data from mitochondrial (mt) gene fragments (e.g., 16S and *cox1*) provide a wealthy resource for assessing the genetic variations and phylogenetic signals among closely related species of diverse molluscan taxa (Boudry et al., 2003; Park and Kim, 2003; Lam and Morton, 2006; Reece et al., 2008). The present study examined the genetic variations and relationships among three Korean *Thais* species based on partial nucleotide sequences of mt *cox1* gene fragments. In addition, the sequence data presented here provides a very useful molecular identification tool for the three Korean *Thais* species, which are often taxonomically complicated when based upon the morphological characteristics alone.

MATERIALS AND METHODS

The specimens were collected from the intertidal and/or subtidal zones in nine localities of the Korean seashore. Voucher specimens used for species identification and subsequent molecular sequencing were deposited in the Marine Mollusk Resource Bank of Korea (MMRBK; Chungbuk National University, Korea). Identification of the *Thais* specimens was carried out based on the shell characteristics, from

which they were assigned to three species: *T. clavigera*, *T. luteostoma*, and *T. bronni* (Fig. 1). A total of 46 specimens sampled from nine localities representing three Korean *Thais* species were used for sequencing analysis. The total genomic DNA was extracted using a tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. A universal primer set (LCO1490: 5'-GGTCAACAAATCAT AAAGATATTGG-3', HCO2198: 5'-TAAACTTCAGGGT GA CCAAAAAATCA-3') was employed for PCR amplification of the target gene fragment mt *cox1* (Folmer et al., 1994). PCR reactions were performed in a 50 µL reaction volume consisting of 10 units of *Taq* polymerase (Roche, Mannheim, Germany), 2.5 mM dNTP mixture, 2.5 mM MgCl₂ and 20 pmole of each primer with the following amplification conditions: one cycle of the initial denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, primer annealing at 45°C for 30 sec and elongation at 72°C for 1 min with a final extension step at 72°C for 10 min. The PCR-amplified target gene fragment was purified using a QIAquick gel extraction kit (Qiagen) according to the manufacturer's protocol. The sequencing reaction was performed using a BigDye® terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA), and the reaction products were electrophoresed using an ABI 3730XL DNA analyzer. Phylogenetic analysis for the mt *cox1* dataset obtained from the three *Thais* species was performed using different tree-building methods (maximum parsimony [MP], neighbor-joining [NJ], and maximum likelihood [ML]) with PAUP 4.0b10 (Swofford, 2002) using *Thais turbinoides* as an outgroup. NJ and ML analyses were carried out using the HKY+Γ (0.0913 of gamma shape parameter) model that was selected as the 'best-fit' substitution model according to the Akaike information criterion (AIC) from implementation of the Modeltest version 3.7 program (Posada and Crandall, 1998). The reliability of grouping in both MP and NJ methods was estimated using non-parametric bootstrap resampling of 1,000 pseudo-replicates.

RESULTS AND DISCUSSION

Phylogenetic relationships among Korean *Thais* species

The target mt *cox1* gene fragment determined for all *Thais* species is 658 base pairs (bp) in length with no intra- or interspecific length variations. Table 1 lists the GenBank accession numbers for each mt genotype and locality data for the species. The MP analysis of the mt *cox1* dataset showed two distinct clades with very strong support (Fig. 2): one representing the assemblage of mt genotypes from *T. clavigera* and the other containing two groups of mt genotypes,

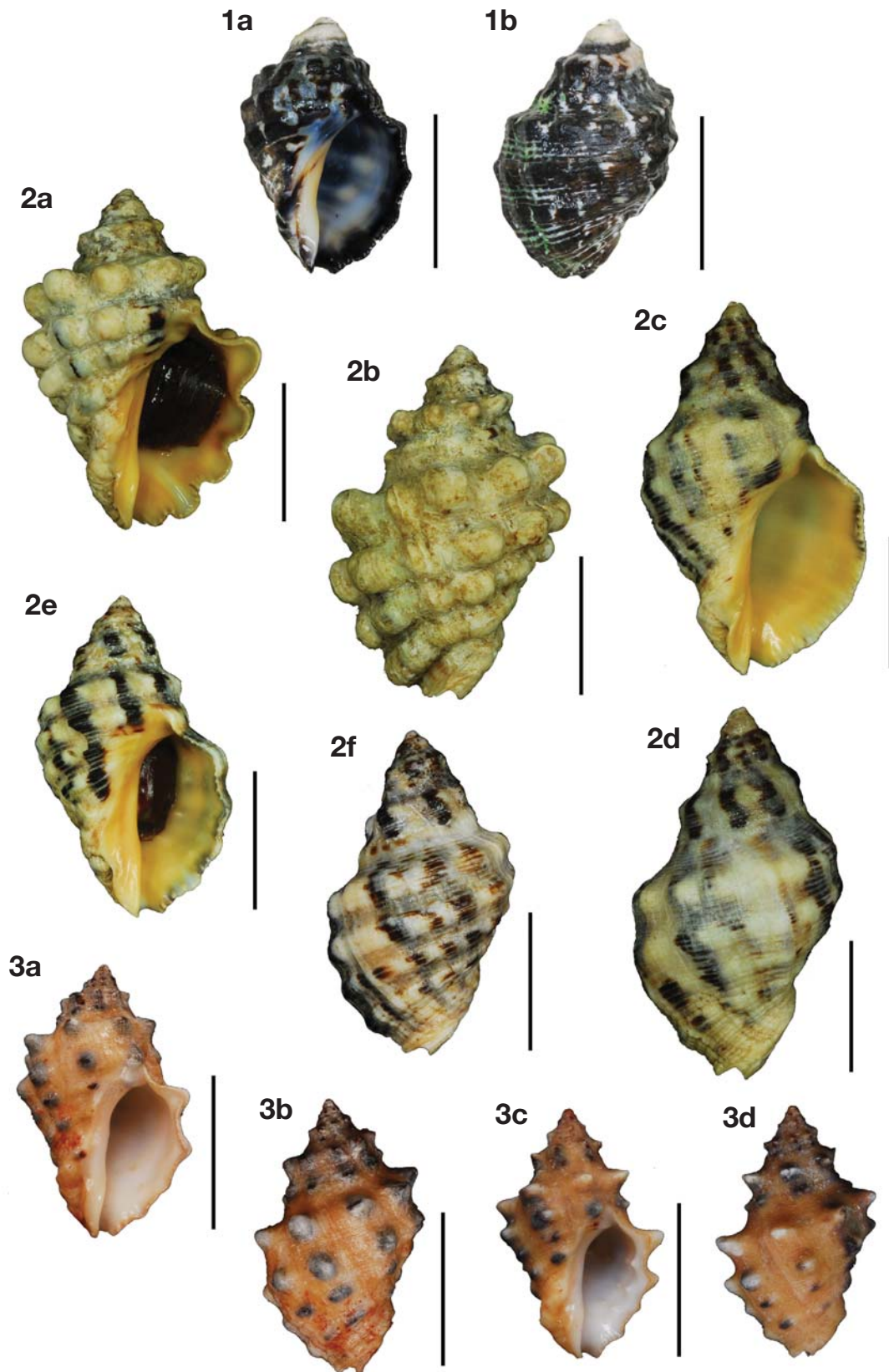


Fig. 1. Shell morphology of three Korean *Thais* species. *T. clavigera* (1a, 1b), *T. bronni* (2a-2f), *T. luteostoma* (3a-3d). Scale bars=2 cm.

Table 1. Geographic origin, sampling locality and GenBank accession numbers for *Thais* species used in this study

Species	Geographic origin	No. of individuals examined	Genotype	GenBank accession no.
<i>Thais luteostoma</i>	Tonggumi, Ulleung-gun Gyeongsangbuk-do	10	TL01 (1)	HQ852742
			TL02 (1)	HQ852743
			TL03 (1)	HQ852744
			TL04 (3)	HQ852745
			TL05 (1)	HQ852746
			TL06 (1)	HQ852747
	Daeyeong, Jeju-do	6	TL10 (1)	HQ852751
			TL12 (1)	HQ852753
			TL02 (1)	HQ852743
			TL04 (1)	HQ852745
			TL07 (1)	HQ852748
			TL08 (1)	HQ852749
<i>Thais bronni</i>	Seogwipo-si, Jeju-do	2	TL10 (2)	HQ852751
	Seogwipo-si, Jeju-do	4	TB01 (1)	HQ852754
			TB02 (1)	HQ852755
			TB03 (1)	HQ852756
	Dodong, Yokji-myeon, Tongyeong-si, Gyeongsangnam-do	9	TB04 (1)	HQ852757
			TB03 (1)	HQ852756
			TB05 (1)	HQ852758
			TB06 (1)	HQ852759
			TB07 (1)	HQ852760
			TB08 (1)	HQ852761
<i>Thais clavigera</i>	Gajin-ri, Goseong-gun, Gangwon-do	2	TB09 (1)	HQ852762
			TB10 (2)	HQ852763
			TB11 (1)	HQ852764
	Wonbuk-myeon, Taeon-gun, Chungcheongnam-do	2	TB07 (1)	HQ852760
			TB08 (1)	HQ852761
			TB12 (1)	HQ852765
	Ganwoldo-ri, Seosan-si, Chungcheongnam-do	1	TB13 (1)	HQ852766
			TC01 (1)	HQ852767
			TC02 (1)	HQ852768
	Hoenggando, Chujado, Jeju-do	1	TC03 (1)	HQ852769
			TC04 (1)	HQ852770
			TC03 (1)	HQ852769
<i>Thais haemastoma</i>	Dodong, Yokji-myeon, Tongyeong-si, Gyeongsangnam-do	2	TC04 (1)	HQ852770
			TC05 (1)	HQ852771
			TC06 (1)	HQ852772
	Geado-ri, Taeon-gun, Chungcheongnam-do	7	TC07 (1)	HQ852773
			TC08 (1)	HQ852774
			TC09 (1)	HQ852775
	Gajin-ri, Goseong-gun, Gangwon-do	1	TC10 (1)	HQ852776
				EU073051
				HQ852777
<i>Thais turbinoidea</i>	South of New Caledonia	1		

representing *T. luteostoma* and *T. bronni*. The grouping patterns among the three species in the MP analysis were identical to those of the NJ and ML analyses (not shown). The bootstrap values for these two clades were very high (100% for *T. clavigera* in both MP and NJ methods, and 96% and 100% for *T. bronni*-*T. luteostoma* in MP and NJ analyses, respectively). The branches representing *T. luteostoma* (92% and 96% bootstrap supports in MP and NJ, respectively) and *T. bronni* (88% and 81% supports in MP and NJ analyses,

respectively) also received relatively strong support. In contrast to the well-structured relationships, the inferred tree showed no particular grouping pattern among the mitochondrial genotypes within each of the three species. Irrespective of the analytical methods (MP, NJ, and ML), the reconstructed phylogenetic trees consistently depicted *T. bronni* and *T. luteostoma* as being more closely related to each other than to *T. clavigera*. This sister group relationship was robustly supported by a bootstrap value of 96% (MP) and 100% (NJ)

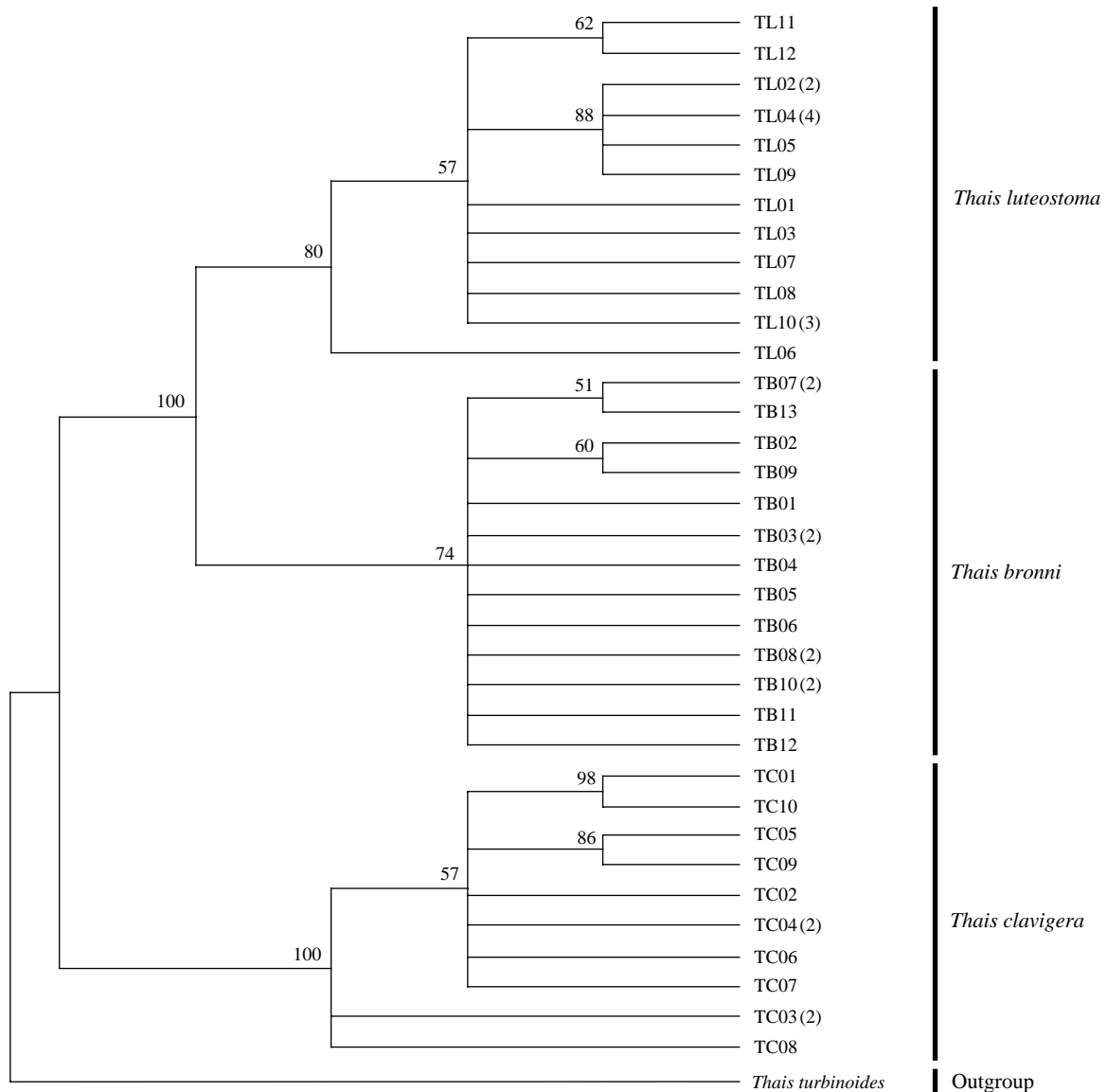


Fig. 2. Bootstrapped consensus tree of 28 equally parsimony trees from heuristic analysis for *cox1* sequence data of three Korean *Thais* species. Bootstrap supporting values from the maximum parsimony method are indicated above the internal branches if $\geq 50\%$. The bootstrap values for each node from neighbor-joining analysis using the HKY+ Γ (0.0913 of gamma shape parameter) model as the 'best-fit' substitution model selected under the Akaike information criterion from the implementation of Modeltest 3.7 program (Posada and Crandall, 1998) were also shown below the branches.

in the different analytic methods (MP and NJ). This result corresponds to the morphological similarities revealed by some earlier studies using comparative analysis of morphological characters (shell, radula, and penis morphology etc.) (Choe and Park, 1997; Tan and Liu, 2001).

Aside from the shell external characteristics, traditional taxonomy has appreciated the radula morphology as one of the most useful features for the classification of a wide range of gastropod groups, including muricid species (Cooke, 1919;

Wu, 1965). Nevertheless, the radula characteristics are often quite similar among closely related congeneric species, making species identification rather difficult based on the radular traits alone (Fujioka, 1985; Tan and Liu, 2001). Some earlier authorities reported that the rachidian teeth of *T. luteostoma* and *T. bronni* were quite similar but noticeably different from those of *T. clavigera* in both the shape and number of denticles. There are usually more than six denticles between the lateral and marginal cusps recorded for the

Korean population of *T. luteostoma* and *T. bronni* (6 and 7-8, respectively) (Choe and Park, 1997). Tan and Liu (2001) observed some variations from Hong Kong population of *T. luteostoma* in the denticle number, ranging from five to eight. The rachidian tooth of *T. clavigera* has fewer denticles (normally 3-4 denticles between lateral and marginal cusps). Although variations in radula characteristics according to the local populations are not uncommon, particularly in the denticle number, similarities in the radula characteristics between *T. bronni* and *T. luteostoma* are in line with the close relationship uncovered by genetic analysis of the molecular data in this study. The closer relationship between *T. bronni* and *T. luteostoma* is also supported by a comparison of the penis morphology. Indeed, the morphology of the male reproductive organ is generally considered species-specific and appears to be very useful for species identification in this genus. Exceptions were found for *T. luteostoma* and *T. jubilaea*, whose penis morphology was more or less similar (Tan and Sigurdsson, 1990; Tan and Liu, 2001). Interestingly, the penis morphology of *T. luteostoma* was quite different from that of *T. clavigera*, but similar to that of *T. bronni* from the Korean population. *T. bronni* has a recurved hooklet-shaped penis with a gradually tapered terminal end (Park, 1996) and these characteristics were also found in Hong Kong (Tan and Liu, 2001) and Korean (personal observations) populations of *T. luteostoma*. The morphological similarities of both radula and penis are consistent with the current molecular data, indicating that *T. luteostoma* and *T. bronni* are very closely related. The inclusion of broader taxon sampling will be needed to place the Korean population of species within the phylogenetic framework of the genus.

Mt DNA variations in Korean *Thais* species

A total of 35 mt genotypes were discovered from 46 individuals of nine local populations of three Korean *Thais* species (Aligned *cox1* sequences for *Thais* species are shown in Supplementary data, Appendix 1). Each of these three species included a large number of mt genotypes: 10 for *T. clavigera*, 13 for *T. bronni* and 12 for *T. luteostoma*. Table 2 lists the uncorrected pairwise (*p*) distance among mt genotypes. The sequence divergence of *T. turbinoides* (outgroup species) from Korean species ranged from 15.2% (between TL08 of *T. luteostoma* and *T. turbinoides* pair and between TB07 of *T. bronni* and *T. turbinoides* pair) to 16.9% (between TC01 of *T. clavigera* and *T. turbinoides* pair). The highest interspecific sequence divergence (17.7%) was observed between *T. haemastoma* and the genotype TL11 from *T. luteostoma*. On the other hand, sequence comparison within Korean congeneric species revealed the *T. clavigera* sequences to differ considerably from those of *T. luteostoma* and *T. bronni*, with maximum differences ranging from 7.14% (between TC01

and TL07 pair) to 7.75% (between TC01 and TB06 pair). In contrast, interspecific sequence divergence between *T. luteostoma* and *T. bronni* was relatively very low, ranging from 1.52% (for TL10 and TB03 pair) to 3.19% (for TL05 and TB06 pair). The lowest interspecific difference value (1.52%) detected between *T. luteostoma* and *T. bronni* was even lower than the highest divergence value detected within *T. clavigera* (2.43% between TC01 and TC08). This low divergence value found between *T. luteostoma* and *T. bronni* implies that cladogenetic split of these two species is very young with a very recent common origin. Further molecular survey using different molecular marker is required to fully understand a detailed picture of the origin for their low level of sequence divergence. In addition, sequence comparison of conspecific individuals revealed extensive sequence variations within each of the three species. The intraspecific differences among the *T. clavigera* mt genotypes were remarkably high, with a maximum 2.43% divergence of a pairwise comparison (found between TC01 and TC08). The sequence divergence within species in each of *T. bronni* and *T. luteostoma* was also high: the maximum value was detected between TB06-TB02 and TB06-TB07 pairs in *T. bronni* (1.37%) and between TL05-TL06, TL05-TL07, and TL05-TL12 pairs in *T. luteostoma* (1.37%).

Along with the considerable genetic divergence within species, there were a large number of mt genotypes discovered in each of the three species. Thirty five mt genotypes were detected from 46 individuals of the three species sequenced. Of these, a small fraction of mt genotypes (9 of 35; 25.7% of total genotypes detected) is represented by more than one individual (Table 1, Fig. 2). With the exception of these, all other mt genotypes (26 of 35; 74.3% of total genotypes detected) were presented by a single individual. These exclusive mt genotypes were shown to be unresolved polytomies at the terminal tips in the reconstructed phylogenetic tree (Fig. 2). This unexpectedly high genotypic diversity encountered within each of the three Korean *Thais* species is assumed to be a reflection of the genetic diversity introduced from potential source populations with diverse geographic origins, such as Taiwan, Hong Kong, and many different coastal regions of South China and Japan. Although a large expansion of deep oceanic water is considered an obstacle to the long-distance dispersal of many marine benthic taxa, some coastal invertebrate taxa have crossed this barrier using a prolonged pelagic larval form (Scheltema, 1971, 1986, 1988) or by rafting (passive transportation of sessile-form juveniles attached to drifting objects) (ÓFoighil et al., 1999). In cases of long range dispersion, the juvenile forms of these animal groups may have the potential to transport and establish successful colonies thousands of kilometers away in downstream habitats that are geographically distant from up-

Table 2. Observed nucleotide difference (above diagonal) and uncorrected pairwise (p) distance (below diagonal) among mt genotypes found in the present study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 TL01	–																	
2 TL02	0.61	–																
3 TL03	0.46	0.76	–															
4 TL04	0.46	0.15	0.61	–														
5 TL05	1.06	0.76	1.22	0.61	–													
6 TL06	0.61	0.91	0.76	0.76	1.37	–												
7 TL07	0.61	0.91	0.76	0.76	1.37	0.91	–											
8 TL08	0.46	0.76	0.61	0.61	1.22	0.76	0.76	–										
9 TL09	0.61	0.30	0.76	0.76	0.15	0.76	0.91	0.91	–									
10 TL10	0.15	0.46	0.30	0.30	0.91	0.46	0.46	0.30	0.46	–								
11 TL11	0.61	0.91	0.76	0.76	1.37	0.91	0.91	0.76	0.91	0.46	–							
12 TL12	0.61	0.91	0.76	0.76	1.37	0.91	0.91	0.76	0.91	0.46	0.61	–						
13 TB01	1.98	2.28	2.13	2.13	2.74	1.98	2.13	2.13	2.28	1.82	2.28	2.28	–					
14 TB02	2.28	2.58	2.43	2.43	3.04	2.28	2.43	2.13	2.58	2.13	2.58	2.58	0.91	–				
15 TB03	1.67	1.98	1.82	1.82	2.43	1.67	1.82	1.82	1.98	1.52	1.98	1.98	0.30	0.61	–			
16 TB04	1.82	2.13	1.98	1.98	2.58	1.82	1.98	1.98	2.13	1.67	2.13	2.13	0.46	0.76	0.15	–		
17 TB05	2.13	2.43	2.28	2.28	2.89	2.13	2.28	1.98	2.43	1.98	2.43	2.43	0.76	1.06	0.46	0.61	–	
18 TB06	2.43	2.43	2.58	2.58	3.19	2.43	2.58	2.58	2.74	2.28	2.74	2.74	1.06	1.37	0.76	0.91	1.22	–
19 TB07	2.28	2.58	2.43	2.43	3.04	2.28	2.43	2.43	2.58	2.13	2.58	2.58	0.91	1.22	0.61	0.76	1.06	1.37
20 TB08	1.82	2.13	1.98	1.98	2.58	1.82	1.98	1.98	2.13	1.67	2.13	2.13	0.76	1.06	0.46	0.61	0.91	1.22
21 TB09	1.98	2.28	2.13	2.13	2.74	1.98	2.13	2.13	2.28	1.82	2.28	2.28	0.61	0.91	0.30	0.46	0.76	1.06
22 TB10	1.98	2.28	2.13	2.13	2.74	1.98	2.13	2.13	2.28	1.82	2.28	2.28	0.61	0.91	0.30	0.46	0.76	1.06
23 TB11	1.98	2.28	2.13	2.13	2.74	1.98	2.13	2.13	2.28	1.82	2.28	2.28	0.61	0.91	0.30	0.46	0.76	1.06
24 TB12	1.82	2.13	1.98	1.98	2.58	1.82	2.13	1.98	2.13	1.67	2.13	2.13	0.76	1.06	0.46	0.61	0.91	1.22
25 TB13	1.82	2.13	1.98	1.98	2.58	1.82	1.98	1.98	2.13	1.67	2.13	2.13	0.46	0.76	0.15	0.30	0.61	0.91
26 TC01	6.84	6.84	6.69	6.69	6.99	6.84	7.14	6.99	6.54	6.69	6.84	6.84	7.30	7.60	6.99	7.14	7.14	7.75
27 TC02	5.93	6.23	5.78	6.08	6.38	5.93	6.23	6.08	5.93	5.78	5.93	5.93	6.38	6.69	6.08	6.23	6.23	6.84
28 TC03	5.62	5.93	5.78	5.78	5.93	5.62	5.93	5.78	5.62	5.47	5.62	5.62	5.78	6.08	5.47	5.62	5.62	6.23
29 TC04	6.08	6.38	5.93	6.23	6.54	6.08	6.38	6.23	6.08	5.93	6.08	6.08	6.54	6.84	6.23	6.38	6.38	6.99
30 TC05	5.78	6.08	5.62	5.93	6.54	5.78	6.08	5.93	5.78	5.62	5.78	5.78	6.54	6.84	6.23	6.38	6.38	6.99
31 TC06	6.08	6.38	5.93	6.23	6.54	6.08	6.38	6.23	6.08	5.93	6.08	6.08	6.54	6.84	6.23	6.38	6.38	6.99
32 TC07	5.78	6.38	5.93	6.23	6.54	5.78	6.38	6.23	6.08	5.93	6.08	6.08	6.54	6.84	6.23	6.38	6.38	6.99
33 TC08	6.23	6.54	6.38	6.38	6.69	6.23	6.23	6.38	6.23	6.08	5.93	6.23	6.38	6.69	6.08	6.23	6.23	6.84
34 TC09	5.62	5.93	5.47	5.78	6.38	5.62	5.78	5.78	5.62	5.47	5.62	5.62	6.08	6.38	5.78	5.93	5.93	6.54
35 TC10	6.23	6.23	6.08	6.08	6.38	6.23	6.54	6.38	5.93	6.08	6.23	6.23	6.69	6.99	6.38	6.54	6.54	7.14
36 <i>Thais haemastoma</i>	16.72	16.72	16.57	16.57	17.02	16.57	16.87	16.57	16.72	16.57	17.74	17.02	17.02	16.87	16.72	16.87	16.57	17.17
37 <i>Thais turbinoides</i>	15.65	15.81	15.35	15.65	15.65	15.35	15.65	15.20	15.65	15.50	15.65	15.96	15.81	15.35	15.50	15.65	15.35	15.81

Table 2. Continued

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
1 TL01	15	12	13	13	13	12	12	45	39	37	40	38	40	38	41	43	41	110	103
2 TL02	17	14	15	15	15	14	14	45	41	39	42	40	42	40	42	39	41	110	104
3 TL03	16	13	14	14	14	13	13	44	38	38	39	37	39	37	39	41	40	109	101
4 TL04	15	13	14	14	14	13	13	44	40	38	37	39	41	39	41	42	40	109	103
5 TL05	20	17	18	18	18	17	17	46	42	39	43	43	43	43	44	42	42	112	103
6 TL06	15	12	13	13	13	12	12	45	39	37	40	38	40	38	41	37	41	109	101
7 TL07	16	13	14	14	14	14	13	47	41	39	42	40	42	42	42	38	43	111	103
8 TL08	16	13	14	14	14	13	13	46	40	38	41	39	41	41	42	38	42	109	100
9 TL09	17	14	15	15	15	14	14	43	39	37	40	38	40	40	41	37	39	110	103
10 TL10	14	11	12	12	12	11	11	44	38	36	39	37	39	39	40	36	40	109	102
11 TL11	17	15	15	15	15	14	14	45	39	37	40	38	40	40	39	37	41	110	103
12 TL12	17	14	15	15	15	14	14	45	39	37	40	38	40	40	41	37	41	112	105
13 TB01	6	5	4	4	4	5	3	48	42	38	43	43	43	43	42	40	44	112	104
14 TB02	8	7	4	6	6	7	5	50	44	40	45	45	45	45	44	42	46	111	101
15 TB03	4	3	2	2	2	3	1	46	40	36	41	41	41	41	40	38	42	110	102
16 TB04	5	4	3	3	3	4	2	47	41	37	42	42	42	42	41	39	43	111	103
17 TB05	7	6	5	5	5	6	4	47	41	37	42	42	42	42	41	39	43	109	101
18 TB06	9	8	7	7	7	8	6	51	45	41	46	46	46	46	45	43	47	113	104
19 TB07	—	7	6	6	6	7	3	45	39	35	40	40	40	40	39	37	41	109	100
20 TB08	1.06	—	5	5	5	6	4	47	41	39	42	42	42	42	41	39	43	109	101
21 TB09	0.91	0.76	—	4	4	5	3	48	42	38	43	43	43	43	42	40	44	110	102
22 TB10	0.91	0.76	0.61	—	—	5	3	48	42	38	43	43	43	43	42	40	43	110	102
23 TB11	0.91	0.76	0.61	0.61	0.61	5	3	48	42	38	43	43	43	43	42	40	44	112	103
24 TB12	1.06	0.91	0.76	0.76	0.76	—	4	47	41	37	42	42	42	42	41	41	43	111	103
25 TB13	0.46	0.61	0.46	0.46	0.46	0.61	—	47	41	37	42	42	42	42	41	39	43	109	101
26 TC01	6.84	7.14	7.30	7.30	7.30	7.14	7.14	—	9	12	9	11	9	9	16	12	4	111	111
27 TC02	5.93	6.23	6.38	6.38	6.38	6.23	6.23	1.37	—	7	2	6	2	4	9	7	5	112	106
28 TC03	5.32	5.93	5.78	5.78	5.78	5.62	5.62	1.82	1.06	—	7	9	7	7	12	10	8	111	103
29 TC04	6.08	6.38	6.54	6.54	6.54	6.38	6.38	1.37	0.30	1.06	—	6	2	4	9	7	5	112	106
30 TC05	6.08	6.38	6.54	6.54	6.54	6.38	6.38	1.67	0.91	1.37	0.91	—	6	6	13	3	7	111	104
31 TC06	6.08	6.38	6.54	6.23	6.54	6.38	6.38	1.37	0.30	1.06	0.30	0.91	—	4	9	7	5	112	106
32 TC07	6.08	6.38	6.54	6.54	6.54	6.38	6.38	1.37	0.61	1.06	0.61	0.91	0.61	—	11	7	5	110	104
33 TC08	5.93	6.23	6.38	6.38	6.38	6.23	6.23	2.43	1.37	1.82	1.37	1.98	1.37	1.67	—	14	12	110	108
34 TC09	5.62	5.93	6.08	6.08	6.08	6.23	5.93	1.82	1.06	1.52	1.06	0.46	1.06	1.06	2.13	—	8	112	104
35 TC10	6.23	6.54	6.69	6.69	6.69	6.54	6.54	0.61	0.76	1.22	0.76	1.06	0.76	0.76	1.82	1.22	—	109	107
36 <i>Thais haemastoma</i>	16.57	16.57	16.72	16.72	16.72	16.87	16.57	16.87	17.02	16.87	17.02	16.87	17.02	16.72	16.72	17.02	16.57	—	119
37 <i>Thais turbinoides</i>	15.20	15.35	15.50	15.50	15.50	15.65	15.65	16.87	16.11	15.65	16.11	15.81	16.11	15.81	16.41	15.81	16.26	18.09	—

TL, *Thais luteostoma*; TB, *T. bronni*; TC, *T. clavigera*.

stream source populations using the oceanic current systems. Indeed, *Thais* species have a wide distribution range in the Asian Pacific Ocean from the South China Sea area (including Hong Kong, Taiwan) to Northeast Asia (including Japan and Korea) (Kuroda et al., 1971; Choe and Park, 1997; Tan, 2000; Tan and Liu, 2001). It is possible to envisage that a continuous influx of larval immigrants from their potential source populations might contribute to some extent to the near-shore malacofauna of the Korean Peninsula. Of the western boundary currents in the North Pacific Ocean, the Kuroshio Current System is a major component of the most influential warm current that leaves the east coast of Taiwan. Its surface water effects travel northeastward to many regional rocky shores in eastern China, mainland Japan, and the Korean Peninsula by streams of the Tsushima Current and Yellow Sea Current. These current systems running northeastward may be able to transport planktotrophic larvae or drifting objects that carry sessile forms of life (e.g., egg capsules of *Thais* species) to many different downstream islands and/or continental rocky shores. The routine influx of these types of immigrants from geographically distant source populations with different geographic origins (e.g., Taiwan, Hong Kong, and many different coastal regions of South China and Japan) and their ability to colonize in downstream habitats might act as an effective supplier of new recruitment, thereby shaping the contemporary population structure of Korean *Thais* species. Under this assumption, the mt genotypic diversity found among Korean *Thais* populations is likely to reflect a subset of the genetic diversity of their potential source populations. Sampling from their potential source populations was not included in the present study. Therefore, further comprehensive taxon sampling from unstudied potential source populations will be needed to determine the origin and key factors responsible for the high level of genetic diversity discovered within each of the three Korean *Thais* species examined.

ACKNOWLEDGEMENTS

The authors wish to thank Roland Houart for the *T. turbinoides* specimens. This study was supported by a research grant of the Chungbuk National University in 2009.

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Received January 14, 2011
Accepted March 3, 2011

Supplementary data, Appendix 1. Multiple sequence alignment of *cox1* gene fragments for *Thais* species used in this study

	50	100
TL01	TACATTATATATTTTATTGGAATATGATCAGGCTTAGTAGGTACTGCTTTAAGTCTCCTTATTCGAGCTGAATTAGGACAACCTGGGGCTTTATTAGGT	
TL02
TL03
TL04
TL05
TL06C
TL07G.....
TL08
TL09
TL10
TL11
TL12
TB01C.....
TB02C.....
TB03C.....
TB04C.....
TB05C.....
TB06C.....G.....
TB07C.....
TB08C.....
TB09C.....
TB10C.....
TB11C.....G.....
TB12G.....C.....
TB13C.....C.....
TC01G.....C.....G.....
TC02G.....C.....G.....
TC03G.....C.....G.....
TC04G.....C.....G.....
TC05G.....C.....G.....
TC06G.....C.....G.....
TC07G.....C.....G.....
TC08G.....C.....G.....
TC09G.....C.....G.....
TC10G.....C.....G.....
<i>Thais haemastoma</i>	...G.....G.....T.TC.....C.....C.A.....G.....A.....A.....A.....
<i>Thais turbinoides</i>C.....G.....TC.T.....C.G.....T.....C.....T.G.....A.....C.G.....C.G.....

TL, *Thais luteostoma*; TB, *T. bronni*; TC, *T. clavigera*.

Supplementary data, Appendix 1. Continued

	150	200
TL01	GACGATCAGTTATACAAATGTTATTGTTACAGCACATGCTTTTGTAATAAATTTTTTCTTGTTATGCGCTATGATAATCGGTGGGTTGGTAATTGATTAG	
TL02	
TL03	
TL04	
TL05	
TL06	T.
TL07	
TL08	
TL09	
TL10	
TL11	G.
TL12C	
TB01	C.
TB02	C.
TB03	C.
TB04	C.
TB05	C.
TB06	C.
TB07	C.
TB08A.	C.
TB09	C.
TB10	C.
TB11	C.
TB12	C.
TB13	C.
TC01C	C.
TC02C	C.
TC03	C.
TC04C	C.
TC05C	C.
TC06C	C.
TC07C	T.
TC08C	C.
TC09C	C.
TC10C	C.
<i>Thais haemastoma</i>T.C.....A.....C.....TC.A.....A.....T.A.T.....A.....	
<i>Thais turbinoides</i>T.....C.G.T.C.G.....T.....T.....C.....A.....A.....T.A.T.....A.C.....G.	

TL, *Thais luteostoma*; TB, *T. bronni*; TC, *T. clavigera*.

Supplementary data, Appendix 1. Continued

	250	300
TL01	TTCCCTTTAAATATTAGGAGCTCCCGATATGGCTTTTCCTCGTTTAAATAATATAAGTTTTTGATTATTACCTCCTGGCGCTTTTATTGCTTCTTTCTTCGGC	
TL02
TL03
TL04
TL05C.....
TL06
TL07
TL08C.....
TL09
TL10
TL11
TL12
TB01A.....A.....
TB02C.....A.....A.....
TB03A.....A.....
TB04A.....A.....
TB05A.....A.....
TB06C.....A.....A.....
TB07A.....A.....
TB08C.....A.....A.....
TB09C.....A.....
TB10A.....A.....
TB11A.....A.....
TB12A.....A.....
TB13A.....A.....
TC01G..G..GT.....C.....C.....A.....A.....
TC02G.....G.....C.....A.....A.....
TC03G.....G.....C.....A.....A.....
TC04G.....G.....C.....A.....A.....
TC05G.....G.....C.....A.....A.....
TC06G.....G.....C.....A.....A.....
TC07G.....G.....C.....A.....A.....
TC08G.....G.....A.....A.....C.....
TC09G.....G.....C.....A.....A.....
TC10G.....G.....C.....A.....A.....
<i>Thais haemastoma</i>C.T..GC.....T.....A.....TT.A.....C.....
<i>Thais turbinoides</i>C.A.....G..G..A..T.....G.....TT.AC..C.T.....

TL, *Thais luteostoma*; TB, *T. bronni*; TC, *T. clavigera*.

Supplementary data, Appendix 1. Continued

	350	400
TL01	TGCAGTAGAGAGAGGGGTAGGGACCGGATGAACGGTGTATCCTCCGTTGGCCGGAATCTGGCCCATGCTGGTGGCTCAGTAGACCTTGCAATTTTCT	
TL02C.....	T.....
TL03	T.....
TL04C.....	T.....
TL05C.....	T.....
TL06	T.....
TL07G.....	T.....
TL08	T.....T.....
TL09G.....C.....	T.....
TL10	T.....
TL11	T.....
TL12	T.....
TB01G.....T.....	T.....
TB02	C.....G.....G.....	T.....
TB03G.....G.....	T.....
TB04G.....G.....	T.....
TB05G.....G.....T.....	T.....T.....
TB06G.....G.....T.....	T.....
TB07G.....G.....T.....	T.....
TB08G.....G.....T.....	T.....
TB09G.....G.....T.....	T.....
TB10G.....G.....T.....C.....	T.....
TB11G.....G.....T.....	T.....
TB12G.....G.....T.....	T.....
TB13G.....G.....T.....	T.....
TC01G.G.A.....G.....T.....A.....T.....	T.....
TC02G.G.A.....G.....C.....A.....T.....	T.....
TC03G.G.A.....G.....T.....A.....T.....	T.....
TC04G.G.A.....G.....T.....A.....T.....	T.....
TC05G.G.A.....G.....T.....A.....T.....	T.....
TC06G.G.A.....G.....T.....C.....A.....T.....	T.....
TC07G.G.A.....G.....T.....A.....T.....	T.....
TC08G.G.A.....G.....T.....A.....T.....	T.....
TC09G.G.....G.....T.....A.....T.....	T.....
TC10G.G.A.....G.....T.....A.....T.....	T.....
<i>Thais haemastoma</i>	T.....A.....A.T.A.A.G.....T.T.....T.A.T.....A.....C.A.T.....T.....C.....A	
<i>Thais turbinoides</i>	...T.G.A.....T.A.....G.T.....TC.A.T.....T.A.T.C.C.....T.G.....T.....T.....	

TL, *Thais luteostoma*; TB, *T. bronni*; TC, *T. clavigera*.

Supplementary data, Appendix 1. Continued

	450	500
TL01	TTACATTTAGCTGGGGTTTCCTCTATTTTATAGGGGCTGTAAACTTTTATTACAACCATTTATTAATATGCGCTGACGGGGTATACAGTTTGAACGACTTCCTC	
TL02	
TL03T.....	
TL04	
TL05	
TL06T.....	
TL07	
TL08	
TL09	
TL10	
TL11	C.....	
TL12	C.....	
TB01T.....	
TB02T.....	
TB03T.....	
TB04	A.....T.....	
TB05T.....G.....	
TB06T.....C.....	
TB07A.....T.....	
TB08T.....	
TB09	T.....T.....	
TB10T.....	
TB11T.....	
TB12T.....	
TB13T.....	
TC01	C.....G.....C.....A.....T.....A.....	
TC02	C.....G.....C.....A.....T.....A.....	
TC03	C.....G.....C.....A.....T.....A.....	
TC04	C.....G.....C.....A.....T.....A.....	
TC05	C.....G.....C.....A.....T.....A.....	
TC06	C.....G.....C.....A.....T.....A.....	
TC07	C.....G.....C.....A.....T.....A.....	
TC08	C.....G.....C.....A.....T.....A.....G.....	
TC09	C.....G.....C.....A.....T.....A.....	
TC10	C.....G.....C.....A.....T.....A.....	
<i>Thais haemastoma</i>	C.....C.....T.....T.....C.....A.....T.....T.....G.....	C.....
<i>Thais turbinoides</i>	G.....A.....G.....T.....T.....C.....T.....T.....G.....A.....G.....A.....CT.G.....	

TL, *Thais luteostoma*; TB, *T. bronni*; TC, *T. clavigera*.

Supplementary data, Appendix 1. Continued

	550	600
TL01	TTTTCGTATGGTCTGTAAAAATTACAGCGATCCTTCTTCTACTATCTCTCCAGTGTAGCAGGTGCTATTACAATGCTGTTAACAGATCGAAATTTAA	
TL02C.....T.....	
TL03G.....	
TL04T.....	
TL05T.....G.....	
TL06G.....	
TL07G.....	
TL08	
TL09T.....	
TL10	
TL11	
TL12A.....	
TB01	
TB02A.....	
TB03	
TB04	
TB05	
TB06C.....A.....	
TB07	
TB08	
TB09	
TB10	
TB11	
TB12C.....	
TB13	
TC01T.G.A...G...A...T...C...G...G.AT...	
TC02T.G.A...G...A...G...G...G.A...	
TC03T.G.A...A...A...G...G...G.A...	
TC04T.G.A...G...A...G...G...G.A...	
TC05C.T.G.A...G...A...G...G...A...	
TC06T.G.A...G...A...G...G...G.A...	
TC07T.G.A...G...A...G...G...G.A...	
TC08T.G.A...A...A...G...G...G.A...	
TC09T.G.A...G...A...G...G...A...	
TC10T.G.A...G...A...T...C...G...G.A...	
<i>Thais haemastoma</i>T.T.A.A.T...T.A.T...T.A.C...T.A.T.T.T.T.A...T...T...T...	
<i>Thais turbinoides</i>A.T...G.AG...T.GT.GT...T.CT...T...T.T.A...C...	

TL, *Thais luteostoma*; TB, *T. bronni*; TC, *T. clavigera*.

Supplementary data, Appendix 1. Continued

	650	658
TL01	TACTGCATTCTTTGACCCCTGCAGGAGGAGGGGATCCTATTTTATATCAGCATTATTT	
TL02
TL03
TL04
TL05G.....
TL06
TL07
TL08
TL09
TL10
TL11T.....
TL12
TB01C.....C.....C.....
TB02C.....
TB03C.....
TB04C.....
TB05C.....
TB06C.....
TB07C.....
TB08C.....
TB09C.....
TB10C.....
TB11G.....C.....
TB12C.....
TB13C.....
TC01T.....
TC02T.....
TC03T.....T.....
TC04T.....
TC05T.....C.....
TC06T.....
TC07T.....
TC08T.....
TC09T.....C.....
TC10T.....
<i>Thais haemastoma</i>	...G..G..T..C..T...T...G..T...G..T...C.....C.....C.....
<i>Thais turbinoides</i>	...A..G.....A.....T...A...A..C.....C.....C.....

TL, *Thais luteostoma*; TB, *T. bronni*; TC, *T. clavigera*.